



The evolutionary history of vertebrate cranial placodes – I: Cell type evolution

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ABSTRACT

Vertebrate cranial placodes are crucial contributors to the vertebrate cranial sensory apparatus. Their evolutionary origin has attracted much attention from evolutionary and developmental biologists, yielding speculation and hypotheses concerning their putative homologues in other lineages and the developmental and genetic innovations that might have underlain their origin and diversification. In this article we first briefly review our current understanding of placode development and the cell types and structures they form. We next summarise previous hypotheses of placode evolution, discussing their strengths and caveats, before considering the evolutionary history of the various cell types that develop from placodes. In an accompanying review, we also further consider the evolution of ectodermal patterning. Drawing on data from vertebrates, tunicates, amphioxus, other bilaterians and cnidarians, we build these strands into a scenario of placode evolutionary history and of the genes, cells and developmental processes that underlie placode evolution and development.

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Introduction

The ectodermal sensory placodes found in the head of all vertebrate embryos have intrigued evolutionary biologists for decades. Several factors contribute to this fascination. Placodes have been considered to be vertebrate innovations, historically presumed to be lacking in the closest living relatives of the vertebrates (Northcutt, 2005; Northcutt and Gans, 1983). The cells and structures they give rise to are generally involved with cranial sensory reception – sight, olfaction, hearing and gustation – and the relay of these senses to the brain. Huge elaboration of these senses has been suggested to have evolved during the adoption of an active predatory lifestyle in the early vertebrate lineage (Northcutt and Gans, 1983). There is also an anthropocentric fascination, in that we are descended from the lineage in which these evolutionary changes took place.

In this review, we will discuss the evolutionary origin of placodes. We will first briefly introduce vertebrate placodes and their derivatives, we will then sketch the phylogenetic context and consider historical views and hypotheses on placode evolution. The main part of this review will focus on the evolution of placodal cell types. In an accompanying review (Schlosser et al., this issue)

we also consider how ectodermal patterning mechanisms along the dorsoventral and anteroposterior axis were modified during placode evolution. We conclude by suggesting future research challenges and opportunities.

Placodes and their derivative cell types in vertebrates

Here we will only provide a very brief overview of the various placodes and their derivatives (Fig. 1). For more details see recent reviews (Baker and Bronner-Fraser, 2001; Grocott et al., 2012; Schlosser, 2010). The *adenohypophyseal placode* gives rise to the anterior pituitary, the major hormonal control organ of vertebrates with six types of endocrine cells: gonadotropes (luteinising hormone – LH – and follicle-stimulating hormone – FSH), thyrotropes (thyroid-stimulating hormone – TSH), corticotropes (adrenocorticotrophic hormone – ACTH), melanotropes (melanocyte-stimulating hormone – MSH), lactotropes (prolactin – PRL), and somatotropes (growth hormone – GH). The *olfactory placode* generates the chemosensory neurons of the olfactory epithelium and the vomeronasal organ. These are primary sensory cells (i.e. with an axon). Olfactory sensory neurons form a heterogeneous population; cells located in distinct regions of the olfactory epithelium express different olfactory receptor genes, and each cell expresses one of many genes. A number of non-neural cells are also generated in the placode-derived olfactory epithelium. These include sustentacular cells and Bowman secretory cells.

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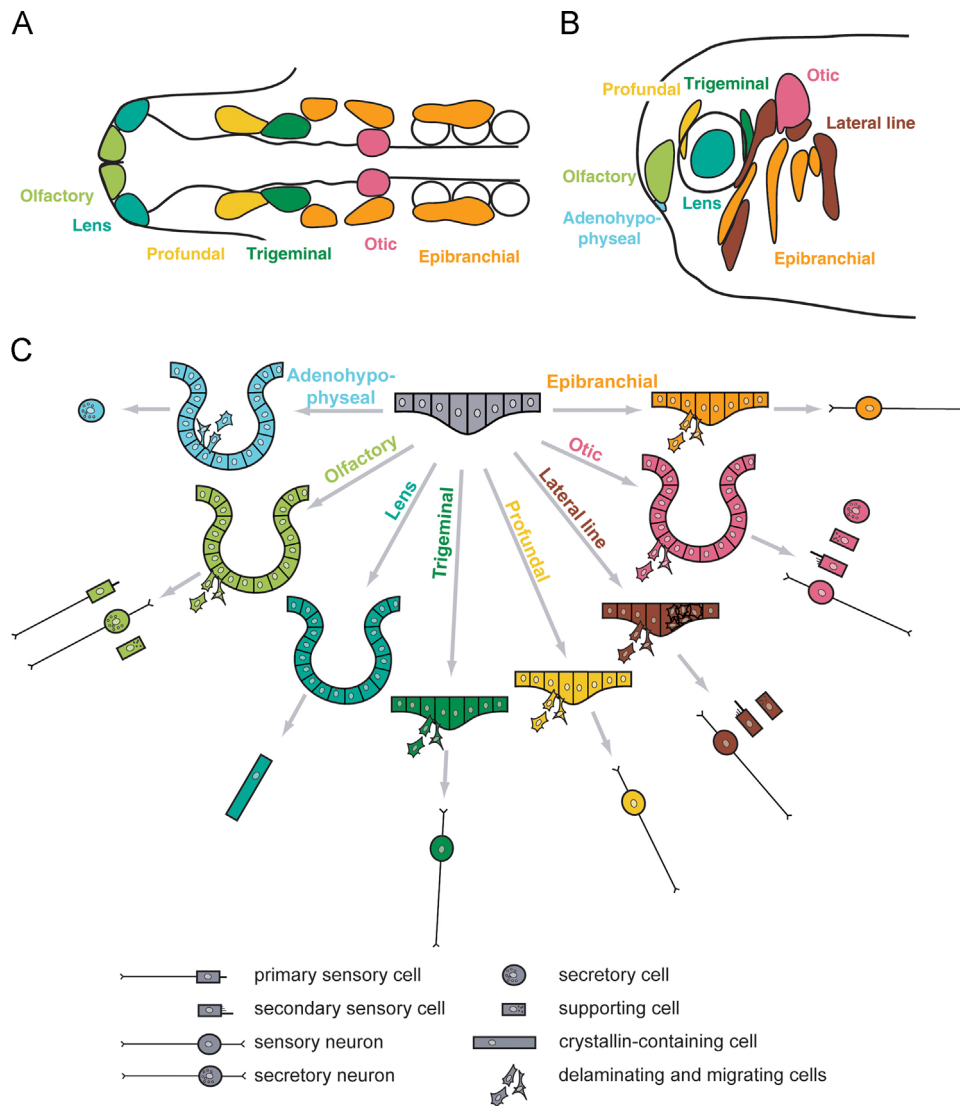


Fig. 1. Vertebrate cranial placodes. (A) Chick embryo (modified from Streit, (2004)). (B) *Xenopus* embryo (modified from Schlosser and Northcutt, (2000)). (C) Developmental fates and derivative cell types of different cranial placodes (modified from Schlosser, (2005)).

Finally, a variety of neuronal cells leave the olfactory epithelium and migrate along the olfactory nerve to colonise pre-optic and hypothalamic parts of the forebrain. The most well-known are the neurons producing gonadotropin releasing hormone (GnRH) which control the secretion of gonadotropins, and the neuropeptide Y (NPY) neurons which in turn regulate GnRH secretion. The *lens placode* will develop into cells which become translucent by accumulation of crystallins and form the lens of the eye.

The *profundal* and *trigeminal* placodes (=ophthalmic and maxillomandibular trigeminal placodes of amniotes) generate somatosensory neurons (SSNs) mediating temperature, touch and pain sensation in the head. The *otic placode* generates mechanosensory hair cells (secondary sensory cells, i.e. without an axon) and the afferent neurons innervating them as well as supporting cells of the inner ear. There are different groups of hair cells in the inner ear, which transmit auditory (hearing) and vestibular (balance) information, respectively. *Lateral line* placodes generate very similar hair cells and afferent neurons of the lateral line system used to detect water movements in many aquatic vertebrates. In some groups they also give rise to modified hair cells, which act as electroreceptors. The *epibranchial* placodes generate viscerosensory neurons (VSNs), which as their name indicates innervate sensory organs associated with the digestive tract and its derivatives. The VSNs innervate, for example,

taste buds in the mouth cavity and pharynx, as well as chemosensory cells in the lung and gut. In addition, the VSNs mediate mechanosensation in the viscera and the afferent arm of cardio-respiratory reflexes, for example O_2 sensing by the gill epithelia in fish and carotid and aortic bodies in terrestrial vertebrates. The VSNs mediate signals initially sensed by endoderm- or neural crest-derived specialized sensory cells, or via free terminal endings. A relatively small placode, the *paratympanic organ placode* has recently been identified in amniotes. This placode generates hair cell-like mechanoreceptors of the paratympanic organ in the middle ear, the homologue of the spiracular organ associated with the first pharyngeal cleft in anamniotes. Afferent neurons to the paratympanic organ hair cells are also generated by the same placode and are located in the geniculate ganglion and a small, separate ganglion (O'Neill et al., 2012). Finally, a series of *hypobranchial* placodes lying ventral to the epibranchial placodes have been described in amphibians (Schlosser, 2003; Schlosser et al., 1999; Schlosser and Northcutt, 2000). What neuronal cell types these placodes generate is at present not clear.

Despite these rather diverse fates, the development of different placodes is similar in a number of respects. First, all but the lens and adenohypophyseal placodes, give rise to some type of sensory receptors and/or neurons (Lassiter et al., 2014; Maier et al., 2014; Piotrowski and Baker, 2014). Second, all placodes undergo some

kind of morphogenetic movements during their development including partial or complete invagination (adenohypophyseal, olfactory, lens and otic placode) and/or migration of sensory or neuronal precursor cells (all except lens placode). Finally, the development of placodes is intimately intertwined with the development of the adjacent neural crest, which guides proper separation and positioning of different placodes and cooperates with placodal cells during ganglion formation (Begbie and Graham, 2001; Coppola et al., 2010; Freter et al., 2013; Shiao and Bronner-Fraser, 2009; Steventon et al., 2014; Theveneau et al., 2013). In addition, all glial cells associated with placodally-derived neurons or sensory cells are now known to be neural crest-derived (Barraud et al., 2010; D'Amico-Martel and Noden, 1983).

Placode evolution – the phylogenetic context

Living vertebrates share a very similar suite of placodes. The earliest diverging extant vertebrate groups are the jawless fishes, the lampreys and hagfishes (Shimeld and Donoghue, 2012), which both have essentially a complete set of placodes. Placed in phylogenetic context (Fig. 2) this means that most placodes had evolved as discrete, clearly identifiable structures prior to the radiation of living vertebrates. Two other lineages of living chordates predate this radiation, the tunicates (sea squirts and allies) and cephalochordates (amphioxus and allies), know collectively by the paraphyletic term protochordates. Historically the cephalochordates have been considered the vertebrates' closest relatives, based principally on morphological characters perceived to be lacking in tunicates. However molecular phylogenetic analyses clearly place the tunicates as sister group to the vertebrates ((Delsuc et al., 2006), Fig. 2). While this implies some chordate characters (such as segmented muscle blocks and organiser-based early embryo patterning) have been lost by tunicates, when considering placode evolution it allows to account for several traits shared by tunicates and vertebrates but not amphioxus, as we discuss below.

Chordates are in the Deuterostomia, a clade they share with the Hemichordata, Echinodermata and possibly the Xenoturbellomorpha. The other bilaterally symmetrical invertebrates comprise the Protostomia, which fall into two broad superphyla, the Lophotrochozoa and Ecdysozoa, both diverse groups encompassing several phyla. The remaining phyla are more distantly related to these bilaterians, with Cnidaria as the sister lineage to the Bilateria

(Fig. 2). When considering the evolution of any character it should be noted that all living species have been evolving for the same length of time; there is no such thing as a living ancestor. However this does not mean all characters change at the same rate, as different lineages may differently preserve characters. With respect to chordates, although tunicates are more closely related to vertebrates, some evidence suggests cephalochordates and vertebrates have, generally speaking, preserved more primitive anatomical, developmental and genomic characters while the rapidly evolving tunicates often display a uniquely derived condition (Paps et al., 2012; Putnam et al., 2008; Yu et al., 2007). However, such generalisations are dangerous when considering individual structures like placodes, since patterns of lineage-specific modifications differ for each individual character. As we will argue in our accompanying review (Schlosser et al., this issue), tunicates despite their highly specialized mode of development actually share some aspects of ectodermal patterning with vertebrates but not amphioxus suggesting that comparisons between tunicates and vertebrates will to some extent allow us to reconstruct evolutionary changes during the transition from ancestral chordates to the tunicate-vertebrate ancestor.

Fossils provide an additional route to understanding the timing of character evolution. Vertebrate embryos fossilise poorly, and hence we should perhaps not realistically expect to directly view transient embryonic structures such as placodes in the fossil record. However the adult structures to which they contribute sometimes do leave traces in fossils: cranial nerves and ganglia may leave telltale marks in cranial bone, the presence of a sophisticated eye implies a lens, and otic structures may indicate an otic placode. While we will not consider such evidence further here, we note that the recent exploitation of high resolution tomographic reconstruction of vertebrate fossils (Gai et al., 2011) may provide scope for a detailed reconstruction of placode derived structures in chordate evolution.

Homology, innovation and a historical perspective on proposals of placode evolutionary origins

Are placodes a vertebrate innovation, or are there placode homologues in invertebrates? Possibly the answer to both questions is yes, as it depends on how “homology” and “innovation” are defined. Both subjects have attracted extensive debate in the

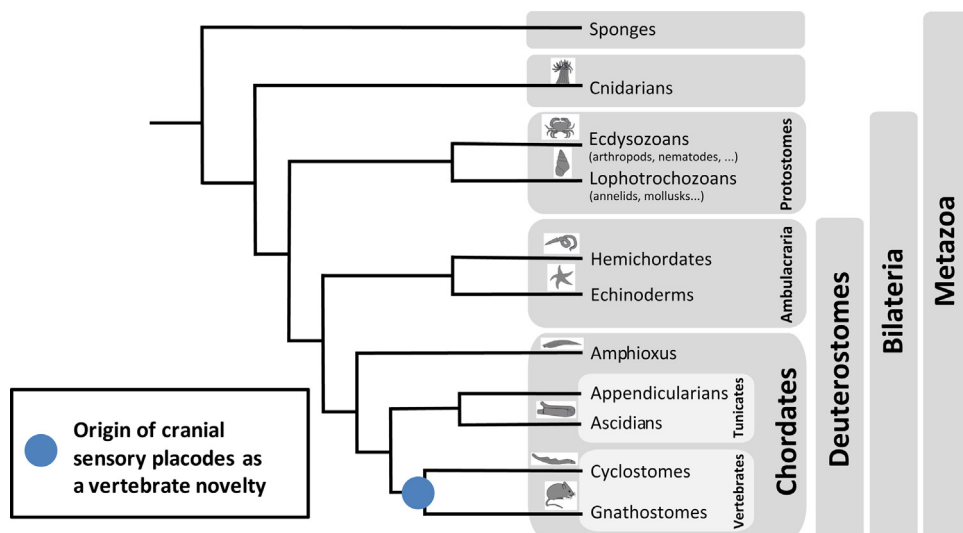


Fig. 2. A simplified phylogeny of the Bilateria, illustrating the relationships of the major taxonomic groups discussed in this paper. Historically, cranial placodes have been considered a vertebrate innovation and are marked as such on this figure. See Fig. 6 in Schlosser et al. (this issue) for a version of this phylogeny on which we have marked evolutionary origins for many of the characters (genes, regulatory interactions, cells and tissues) which together constitute cranial placodes.

Box 1—Homology and Evolutionary Novelties:

Characters in different species are homologous if they are derived from the same character in their last common ancestor (Wagner, 2007). Similarities in homologous characters can therefore be attributed to common ancestry rather than to independent (convergent) adaptive responses to similar environmental challenges. However, homologous characters may have accumulated different heritable variations in different lineages over time and, thus, may appear structurally and functionally quite different from each other. Homology in these cases may only be possible to establish if transitional forms are preserved. A major conceptual problem for this evolutionary notion of homology is how character identity can be recognised across generation boundaries even in the face of heritable variation. In any case character identity implies the conservation of some features (e.g. conservation in position of a character relative to other characters and/or conservation in the relationships among the character's components) despite variation in others and thus can only be recognised for characters possessing a certain complexity and composite nature.

Because characters are complex, composite structures and because parts of these structures may be reshuffled during evolution, homology attributions often hold only for particular levels of comparison. For example, a character may continue to form a conserved part of a larger structure (being homologous in ancestor and descendant), even though its components have been substituted by other (non-homologous) components or the way it is built developmentally has changed in evolution. For example, arthropod segments and the regulatory network of segment-polarity genes establishing these segments have been evolutionarily conserved in spite of wide divergence of upstream generative mechanisms and genes involved (Peel et al., 2005), a phenomenon termed “genetic piracy” or “developmental system drift” (Roth, 1988; True and Haag, 2001). Conversely, a character may have preserved its compositional structure or development (being homologous in ancestor and descendant) but has become redeployed into new (non-homologous) developmental contexts. For example, many signalling pathways have adopted roles in new developmental contexts during evolution; a well studied case is the new role of Hedgehog signalling in butterfly eye spot development (Pires-daSilva and Sommer, 2003).

Homology can only be recognised when characters are preserved in evolution, but occasionally novel characters evolve. It is still disputed, how restrictively or inclusively “Evolutionary Novelty” should be defined (Hallgrímsson et al., 2012; Moczek, 2008; Peterson and Müller, 2013). However, it is generally agreed that only qualitatively novel characters (rather than mere quantitative modifications of existing characters) should be considered. An influential definition suggests that a “novelty is a structure that is neither homologous to any structure in the ancestral species nor homonomous to any other structure of the same organism” (Müller and Wagner, 1991). It is important to realise that typically such absence of homology will apply only to the level of the structure considered – e.g. a new network of regulatory interactions between patterning mechanisms and cell type specific differentiation gene batteries. Since evolution does not operate in a void but “tinkers” with what is already there, new structures are usually built out of old components (such as pre-existing cell types, signalling pathways or local patterning mechanisms) and may be embedded into pre-existing higher order structures (such as global patterning systems). Even for novel structures, we therefore expect to find homologous components and possibly a homologous address in a conserved global coordinate system in species descended from more distant ancestors preceding the origin of the novelty. The recognition of homologous parts or addresses in lineages distantly related to a particular group, therefore, is fully compatible with the origin of a novelty in the

group's last common ancestor due to establishment of new regulatory relationships between them.

literature and we cannot provide an extensive review of these debates here. For a synthesis of what we consider the interface between homology and innovation to be, please see Box 1. In simplified form, homology is derivation of a character in different lineages from the same character in the last common ancestor of the lineages under comparison. However, as characters in this context can be a host of different morphological (for example cell types, organs) or other (genes, regulatory interactions) features, use of the terms “homologue” or “novelty” needs qualification with level, that is explicit description of what type of character is proposed as homologous or novel. Structures such as the vertebrate limb or placodes, for example, may be evolutionary novelties even though many of their cellular or molecular components have homologues in invertebrate taxa.

Over the last 150 years or so, various authors have speculated on the evolutionary history of placodes (see Schlosser, 2005 for review). Table 1 lists some of the key publications, and summarises their conclusions. Below we consider these in terms of the putative level of homology under investigation. Please refer to the table for additional citations.

Hypotheses for placode evolution based on anatomical, positional and embryological evidence

Early proposals of placode homologues in protochordates were based primarily on adult anatomy, relative position and embryological origin. Both Hatschek's pit (which derives from the pre-oral pit) of amphioxus and the neural complex of ascidian tunicates were thought to be organs formed from two tissue sources, with connection between the CNS and non-CNS components. Hatschek's pit is identifiable in adult amphioxus as an evagination of the roof of the pharynx that makes contact with a small area in the base of the brain, and as such appears similar to the juxtaposition of anterior and posterior lobes of the pituitary in the vertebrate brain (Fig. 3). The ascidian neural complex is composed of a ganglion and closely-associated glandular organ, which connects through to the inner oral siphon by means of a ciliated duct that opens via a funnel shaped vent (Fig. 3). As with Hatschek's pit, the original consideration of this as a pituitary homologue comes from the intimate juxtaposition of neural and secretory structures, and corresponding hypothesised endocrine function.

In a similar vein, Jefferies suggested homology between the atrial siphon primordia of ascidians and the vertebrate otic placode (Jefferies, 1986). This assertion was partially based on his contentious interpretation of a set of fossils known collectively as calcichordates, but also included anatomical and positional evidence in that the atria start (in some species) as paired ectodermal invaginations lying alongside the ascidian equivalent of the hindbrain, both also characters of otic placodes.

Hypotheses for placode evolution based on cell type and cell function

Function is not an indicator of homology. However it may reinforce other lines of evidence. Both Hatschek's pit and the neural complex have been proposed as sites of neuropeptide production, based initially on antibody staining. It has also been shown that the atrium of some adult ascidians includes structures known as cupular organs, cell clusters with sensory cilia encased in a gelatinous dome and presumed to be mechanosensory (Bone and Ryan, 1978). These have hence been likened to vertebrate hair

Table 1
A summary of proposals for homologues of placodes or placode-derivatives in tunicates and amphioxus.

Placode	Authors	Species	Hypothesis/key findings	Comments
Olfactory	de Quatrefages (1845)	Amphioxus	Identification of anterior primary sensory neurons and rostral nerve clusters in adult	Subsequently suggested as possible olfactory or mechanosensory organ (see for example Baatrup (1982), Baker and Bronner-Fraser (1997), Sharman et al. (1999)
	Sharman et al. (1999)	Amphioxus	Expression of <i>Msx</i> in larval rostrum and tentative suggestion of olfactory homologue as per de Quatrefages (1845)	Still lack data connecting larval gene expression and adult neural structures
Adenohypophyseal	Julin (1881)	Ascidians	Comparison of ascidian neural complex with vertebrate pituitary	Subsequently supported by peptide antibody staining Chang et al. (1982) and neuroanatomy Gorbman et al. (1999).
	Hatschek (1881)	Amphioxus	Hatschek's pit as a possible adenohypophysis homologue	
	Nozaki and Gorbman (1992)	Amphioxus	Immunoreactivity for adenohypophyseal hormones in Hatschek's pit	Proposed homology of Hatschek's pit with adenohypophysis
	Burighel et al. (1998), Manni et al. (1999)	<i>Botryllus schlosseri</i> (ascidian)	Delamination of neurons in the ascidian neural complex similar to placode neuron delamination	Proposed homology of ascidian neurohypophyseal duct with adenohypophyseal placode
	Boorman and Shimeld (2002), Yasui et al. (2000), Boorman and Shimeld (2002), Christiaen et al. (2002)	Amphioxus	Expression of <i>Pitx</i> in the pre-oral pit of amphioxus, which gives rise to part of Hatschek's pit	Proposed homology of pre-oral pit with adenohypophysis
Otic/Lateral line		<i>Ciona</i>	Expression of <i>Pitx</i> in ascidian oral siphon rudiment and ciliary funnel	Proposed to support homology of ciliary funnel with adenohypophysis
	Bone and Ryan (1978)	<i>Ciona</i>	Identification of sensory cell clusters in ascidian atrium with some similarity to vertebrate otic/lateral line hair cells	Homology to hair cells questionable as these ascidian cells are primary sensory cells
	Jefferies (1986)	Ascidians	Proposed homology of the ascidian atrial openings with vertebrate otic placode	Based on comparative anatomy integrated with palaeontological data
	Wada et al. (1998)	<i>Halocynthia roretzi</i> (ascidian)	Expression of ascidian <i>Pax2/5/8</i> in atrial primordia compared to orthologue expression in vertebrate otic placode	First use of molecular data to support an hypothesis of placode homology
	Burighel et al. (2003)	Various ascidians	Further characterisation of neural complex and identification of secondary sensory neurons in the coronal organ of the oral siphon of diverse ascidian species	Proposal of homology between coronal organ and otic and lateral line placodes.
	Kourakis et al. (2010), Kourakis and Smith, (2007)	<i>Ciona</i>	Development of ascidian atrial primordium regulated by FGF signalling and details of underlying cell biology	These papers do not propose novel homologies but data are discussed in the context of <i>Ciona</i> placode homologues
Others	Lacalli and Hou (1999)	Amphioxus	Detailed characterisation of sensory neurons in the surface ectoderm	Only scattered cells identified, not placode- like territories
	Mazet et al. (2005)	<i>Ciona</i>	Expression of several placode marker genes in ascidian atrial and oral siphon rudiments	Proposal of two ancestral placode territories.
	Bassham and Postlethwait (2005), Benito-Gutierrez et al. (2005), Kozmik et al. (2007), Mazet et al. (2004)	<i>Oikopleura</i> (non-ascidian tunicate) Amphioxus	Expression of placode marker genes in ectodermal sensory structures and proposition of olfactory and adenohypophyseal homologues Expression of various sensory neuron markers (<i>Coe</i> , <i>Six</i> , <i>Eya</i> , <i>Trk</i>) in scattered amphioxus ectoderm cells	Likely to be sensory neurons as identified by Lacalli and Hou (1999)

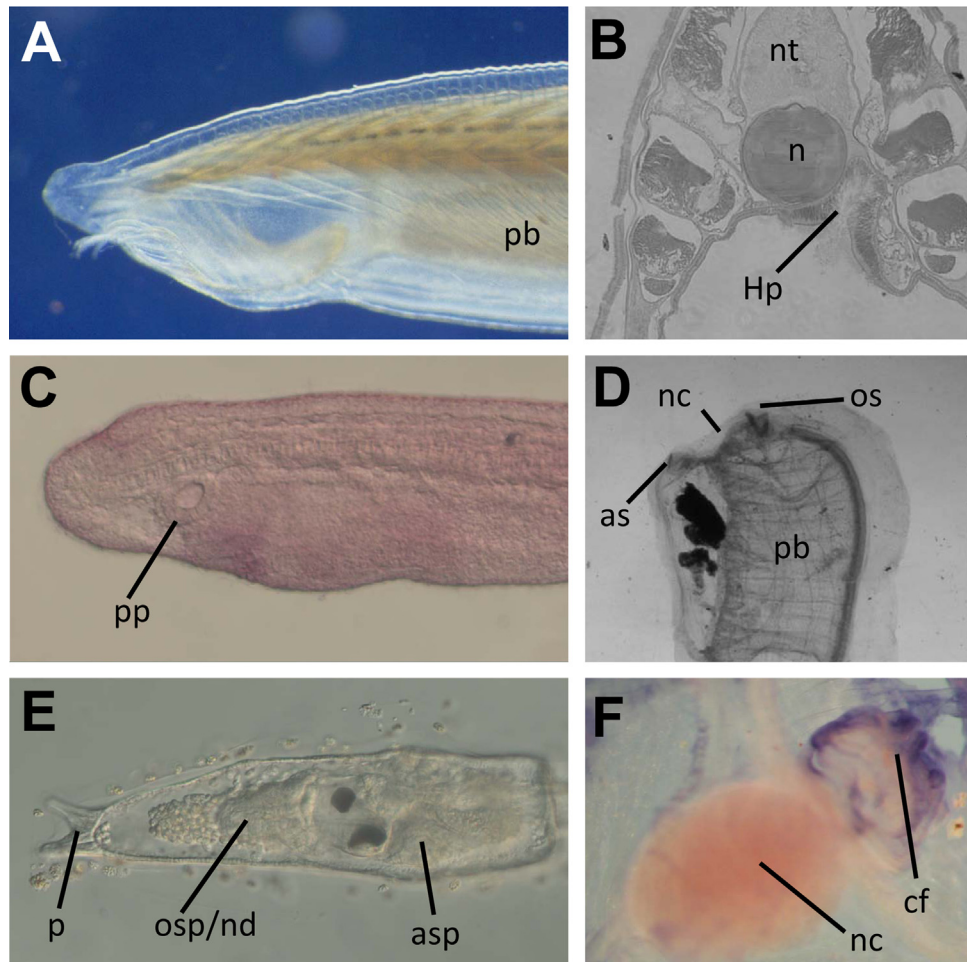


Fig. 3. Some of the protochordate structures hypothesised to be homologous to vertebrate placodes. (A) Head of an amphioxus (*Branchiostoma floridae*), showing lack of obvious cranial sensory organs. pb, pharyngeal basket. (B) Section through the head of an adult amphioxus showing Hatschek's pit (Hp) penetrating up from the pharynx and connecting with the neural tube (nt), n, notochord. (C) Amphioxus larva showing the pre-oral pit (pp). (D) Cleared specimen of the tunicate *Clavelina lepadiformis*, showing the oral (os) and atrial (as) siphons, and the position of the neural complex (nc). (E) Larval head of the tunicate *Ciona intestinalis*, showing the palps (p), the oral siphon primordium/neurohypophyseal duct (osp/nd) and an atrial siphon primordium (asp). (F) Dissected neural complex from *Ciona intestinalis* stained with the Six3/6 orthologue *CiSix3/6*. The neural complex and ciliated funnel (cf) are marked.

cells that form from the otic and lateral line placodes, although their embryonic origin is unknown, and they are primary sensory cells, unlike the secondary mechanosensors that form from the vertebrate otic placode. Further confusing functional interpretation, the oral siphon of ascidians has been shown to include a mechanosensory structure named the coronal organ, which includes genuine secondary sensory cells (Burighel et al., 2003). Several authors have based speculation on placode evolutionary origins on such cell type data (see Table 1 for details).

Amphioxus sensory cells have been described by a number of authors, with the most detailed description by (Lacalli and Hou, 1999). These cells are scattered through the ectoderm rather than focused in organs/ganglia as in vertebrates. The function of these cells has yet to be experimentally verified and most are primary sensory cells although secondary sensory cells have also been described.

Hypotheses for placode evolution based on genes and gene expression

The extension of molecular methods to comparative development and evolution, and the general conservation of genes controlling development across morphologically divergent phyla, stimulated an explosion in the use of gene expression data as test for homology. In 1998 Wada and colleagues showed that the ascidian *Pax2/5/8*

gene, orthologous to the vertebrate otic placode markers *Pax8* and *Pax2*, was expressed in the atrial primordium, in support of Jefferies' hypothesis of homology of these structures (Wada et al., 1998). Similar studies of *Pitx* expression were used as support for the oral siphon as an olfactory/adenohypophyseal placode homologue (Boorman and Shimeld, 2002; Christiaen et al., 2002). Single gene studies like this were then superseded by studies looking at multiple genes known to form a regulatory network in vertebrate placode development, specifically the *Eya* gene, three Six gene families (*Six3/6*, *Six1/2*, *Six4/5*) and two Pax gene families (*Pax6* and *Pax2/5/8*). In ascidians these genes were found expressed in the siphon primordia and cited as further evidence for their homology with placodes (Mazet et al., 2005). Such studies have also suggested, though not very persuasively, that the ascidian palps (an anterior secretory organ that lies just anterior to the oral siphon primordium) may be olfactory homologues. This idea was in part dependent on the early expression of *Eya* in the cells that will give rise to the palps, and also on the identification of sensory neurons amongst the secretory cells (Candiani et al., 2005; Mazet et al., 2005).

Similar studies have also been undertaken in the appendicularian *Oikopleura* and amphioxus and are summarised in Table 1. Many putative placode marker genes have been found to be expressed by scattered ectodermal cells in amphioxus, presumed to be some or

all of the sensory neurons described by (Lacalli and Hou, 1999). While these could be argued as homologous to vertebrate placode derived sensory neurons at one level (i.e. as surface ectoderm-derived sensory cells), the lack of focused domains of cells expressing these genes, as seen for vertebrate placodes and ascidian siphon primordia, does not support homology at the level of a placode. The exception to this may be Hatschek's pit, since the pre-oral pit (the larval structure from which it forms during metamorphosis) does express the pituitary gene *Pitx*, reinforcing homology indicated by anatomical and functional evidence. This, too, however, has its caveats, which we discuss below and in an accompanying paper (Schlosser et al., *this issue*).

Hypotheses for placode evolution based on developmental mechanisms

Assigning homology based on gene expression data, even when addressing suites of genes that interact in other species, is based on correlation. For developmental genes it assumes that their expression in a tissue or cell is conserved which, given the widespread co-option of such genes in animal evolution, and ancestral roles for many genes in ectodermal patterning (see below and Schlosser et al. (*this issue*)), may be an unsafe foundation for evolutionary hypotheses. Understanding of mechanism can provide an additional level of evidence, with the expectation that homology predicts not only expression of genes to be conserved, but also regulation, and function in terms of control of target genes, cell behaviour, differentiation etc.

Our understanding of developmental mechanisms underlying vertebrate placode development is reasonably advanced, however to date only a small number of studies have investigated developmental mechanisms underlying the development of protochordate structures relevant to placode evolution. The development of the pre-oral pit/Hatschek's pit of amphioxus has not been investigated experimentally, while the development of the ascidian oral siphon primordium and the adjacent palps has been subjected to limited study (Wagner and Levine, 2012). Recently, Kourakis and colleagues have undertaken some interesting studies of atrial siphon development (Kourakis et al., 2010; Kourakis and Smith, 2007). Specifically, their work demonstrates a role for FGF signalling in this process, another character in common with otic placode formation, and describes some of the underlying cell biology of atrial siphon invagination. In addition, the gene regulatory networks underlying tunicate development are being worked out at an increasingly detailed level, which will help us to further elucidate homology relationships with vertebrates in the future (Imai et al., 2004, 2006, 2009).

Hypotheses for placode evolution: criticisms and caveats

Combined, these data have been used to support incremental models of placode evolution, with comparison between extant species used to infer ancestral character states. It has, for example, been suggested that the common ancestor of tunicates and vertebrates had two proto-placodal ectodermal domains; one just anterior to the CNS that diversified into the oral siphon in ascidians and anterior placodes in vertebrates, and paired domains parallel to the equivalent of the hindbrain that diversified into the atrial siphons in ascidians and posterior placodes in vertebrates (Graham and Shimeld, 2013). The term 'proto-placodal' is used here and throughout this review in a wider sense than by (Schlosser, 2005) (where it was used to describe the first true placode) to denote regions of the non-neural ectoderm that even though they are not true placodes are positionally homologous to vertebrate placodes and which may have undergone some morphogenetic movements and may have formed some sensory cells. The common ancestor of chordates would be less complex, with

evidence for only an anterior proto-placodal domain, unless one considers the entire posterior ectoderm as a big caudal proto-placode, one interpretation of a study of the regulation of amphioxus posterior ectodermal neurogenesis (Lu et al., 2012). Further diversification of placodes including the origin of, for example, the lens and trigeminal/profundal placode then possibly occurred specifically in the vertebrates lineage (for detailed discussion see (Graham and Shimeld, 2013; Schlosser, 2005)).

Whereas such models present plausible and parsimonious scenarios based on the character distribution in extant taxa, they are always tentative and subject to some caveats. For example we know that some of the gene networks used as evidence for homology are much more ancient than the chordate common ancestor. Their parallel cooption in the different chordate lineages is a real possibility. If we take the putative anterior placodal territory, then some of the genes involved have an ancient role in anterior specification in bilaterians and even aboral specification in cnidarians (Sinigaglia et al., 2013). We discuss this in more detail in our accompanying paper (Schlosser et al., *this issue*).

Prior to developing contact with the base of the brain the amphioxus pre-oral pit appears to be of combined ectodermal and endodermal origin, and it has been suggested that it partly derives from the left anterior gut diverticulum (an endodermal structure) which makes contact and then fuses with the ectoderm (Conklin, 1932). In contrast, the vertebrate adenohypophyseal placode is purely ectodermal. Until recently, the adenohypophysis of hagfishes had been thought to arise from an outgrowth of foregut endoderm (Gorbman, 1983; Gorbman and Tamarin, 1985), lending support to the idea that an ectodermal origin was specific to lampreys and gnathostomes. However, it has now been confirmed that the adenohypophysis in hagfish also has an ectodermal origin as in other vertebrates (Oisi et al., 2013). Fate mapping cells of both ectodermal and endodermal domains in amphioxus would be needed to fully understand the embryonic origin of Hatschek's pit, as currently we do not know which cells contribute to the adult structure. If mixed endodermal and ectodermal origin is confirmed, this would appear to be a major distinction between Hatschek's pit and the vertebrate adenohypophysis. If homology is to be considered in these instances, we also should be able to explain how differences in the tissues of origin for these putative anterior placode homologues evolved.

To integrate such discrepancies into evolutionary hypotheses is not straightforward. At present we lack sufficient data from both protochordate lineages, and we also have to consider how much we weigh differences when compared to similarities. For example the anterior-posterior extent of neurulation is different in vertebrates and ascidians and includes the putative anterior placodal domain in ascidians but not in vertebrates. This appears a major difference at the tissue level. However, until we know the developmental details in both lineages as well as in amphioxus as an outgroup, we cannot infer either the likely ancestral state, or which evolutionary specialisations evolved in each lineage.

To explore this more closely, we will now consider in detail the current state of knowledge of placode cell type evolution, including an assessment of our current knowledge of potentially relevant cells that develop from the ectoderm of tunicates, amphioxus and other bilaterians. In combination with our accompanying paper on ectodermal patterning (Schlosser et al., *this issue*), our aim is to provide as solid a foundation as currently possible for evaluating and revising hypotheses of placode evolutionary history. We will then revisit models of placode evolutionary origins, attempting to deconstruct the many placode characters and trace the evolution of each. In doing so, we aim to highlight both the ancestry of placode characters, and where innovation can be identified in the form of new characters and new character combinations.

An evolutionary perspective on placode cell types

Cell types are fundamental units of animal development and evolution. They can act as modules during evolution and might be older than the structures in which they are located. Modifications of the bauplan via changes in early patterning mechanisms and evolution of cell types are complementary aspects of the evolution of structural novelties. In this section we discuss the presence of putative placode-derived cell type homologues in invertebrates and what we have learned from comparative studies of cell types with regard to the evolution of placodes.

Often when talking about cell types we mean terminally differentiated cells. These are present within organs of larvae or adult animals in very complex arrangements, often scattered through epithelia and mesenchymal tissue. Terminally differentiated cells fulfill structural, biochemical and physiological functions and therefore express effector genes and regulators thereof. Progenitor cells transiently present during embryonic development can also be classified as cell types. Progenitors are usually arranged into continuous fields expressing defined sets of transcription factors. Within these fields progenitors, often distributed in a salt-and-pepper manner exit the cell cycle and differentiate into mature cell types depending on their localisation and the regulatory landscape in the cell at the time of differentiation. Several cell types can originate from a common field of progenitors.

It is a matter of convention as to what level of specialisation shall be used as a criterion to identify a cell type. For example, neurons form a large family of cell types, sensory neurons are a subfamily of neurons, viscerosensory neurons are a subgroup thereof and so on down to single cells.

Cell types can be defined by the genes they express, i.e. their transcriptome. However, not even cells of the same type in the same organism will express identical levels of all genes because of stochastic variation as well as temporal variations in transcript levels (Kim and Marioni, 2013; Raj and van Oudenaarden, 2008). A fortiori, the levels of expression of orthologous genes in homologous cells in different species will vary. Moreover, regulatory changes during evolution might result in some new genes being expressed and others to be down-regulated in homologous cells in divergent lineages.

Yet, sets of functionally-related genes, involved in a common molecular process and often co-regulated by a few transcription factors forming gene regulatory networks, are much conserved and can act as a molecular signature for cell types (Arendt, 2008). Such “subroutines” are shared between very different cell types, but in combination define precisely the identity and function of mature cells, at least in neurons (Hobert et al., 2010).

Other characteristics can be used to define cell types, such as ultrastructure. In particular for sensory cells, the number and organisation of cilia and microvilli is used to classify them, and can hint at homology of cell types. In the case of neurons, the presence of processes such as axons and dendrites is cell type-specific, and the connectivity of neural circuits is also an important character to define neuronal types.

The domain of embryonic origin of a cell should not be a criterion to define a cell type because the same cell type can conceivably arise in different parts of the organism. However, common or related embryonic origin is an important factor in assessing, which cells of a similar type are homologous in distant species.

Finally function is an important attribute of cell types, although by itself it is by no means guaranteed that similar cells sharing a function do so because of shared ancestry. Sensory cells transduce a signal from physical stimuli, so electrical or biochemical responses to chemicals, light or mechanical or other stimuli are naturally characteristic of sensory cell types.

Novel or modified cell types can originate essentially in two ways (Fig. 4). The first is by splitting an existing cell type into two sister cell types. Possibly, a multifunctional cell evolves into two cells, each taking over part of the specific genes and functions between the two, according to the division of labour model (Arendt, 2008). In such cases, it is expected that several marker genes will be kept in common between the two daughter cells, while others will be differentially expressed. However, if the separation of functions is dramatic, e.g. if an effector neuron and a sensory cell evolve from a common sensory-motor neuron, sister cells might have more differences than they have in common, making it difficult to recognise the homology. The existence of a common progenitor during development can be a sign of common ancestry. The second way new cell types evolve is by merging parts of the transcriptomes of existing cell types to form a new cell expressing sets of genes in a novel combination. This is equivalent to co-option of gene networks. If expression of only a few genes is recruited or lost, homology to the parental cell types will be relatively easy to recognise since most of the cell type-specific genes will be shared. If on the contrary a large set of genes is co-opted, it will be more problematic to decide to what ancestral cell type the new one is homologous, and indeed cell types can in some cases be considered as novelties although the genes sets that compose them can be more ancient. Both division of labour and gene network co-option involve changes in gene regulation and might or might not involve gene duplication. In the following paragraphs we discuss the evolutionary history of the cell types generated by cranial placodes and how these cell types might have arisen or been recruited during the invertebrate to vertebrate transition.

Olfactory placode

Chemoreception is a very ancient sensory modality that has been described in a wide range of organisms from unicellular eukaryotes to cnidarians, nematodes, insects and vertebrates (Chia and Koss, 1979). Outside vertebrates, olfactory systems have been best studied in nematodes and insects. However, although there are similarities between the olfactory systems of insects and vertebrates in the logic of neuronal connectivity and information processing, the receptors and signal transduction pathways are different (Kaupp, 2010). In vertebrates, the olfactory sensory neurons sense chemicals through olfactory receptors (ORs), a vast sub-family of G-protein coupled receptors. The signal is transduced via olfactory-specific G α proteins encoded by genes of the GNAL and GNAS families (Oka and Korsching, 2011). These promote the production of cAMP which in turn activates the cAMP-gated ion channel CNCG. The chemosensory cells in the vomeronasal organ express the pheromone receptors V1R and V2R, which represent another class of G protein-coupled receptors. These activate a TRP-family ion channel through phospholipase C β signalling, a pathway common to photoreceptors and taste cells.

In insects, olfaction is mediated by receptors not related to vertebrate olfactory, vomeronasal or taste receptors. Ionotropic receptors (referred to as IRs) are derived members of the ionotropic glutamate receptor gene family that appeared early in arthropod evolution and now mediate olfaction in a subset of neurons in insects and the olfactory neurons of at least some crustaceans (Corey et al., 2013). In addition, insects have a number of olfactory receptor genes specific to insects that bear no resemblance to vertebrate olfactory receptors, other than also being seven transmembrane proteins (Kaupp, 2010). ORs in insects are ionotropic but do elicit metabotropic signalling via cAMP, although the target of the secondary messenger might be olfactory receptors themselves (Kaupp, 2010). The olfactory receptors of nematodes are bona fide G

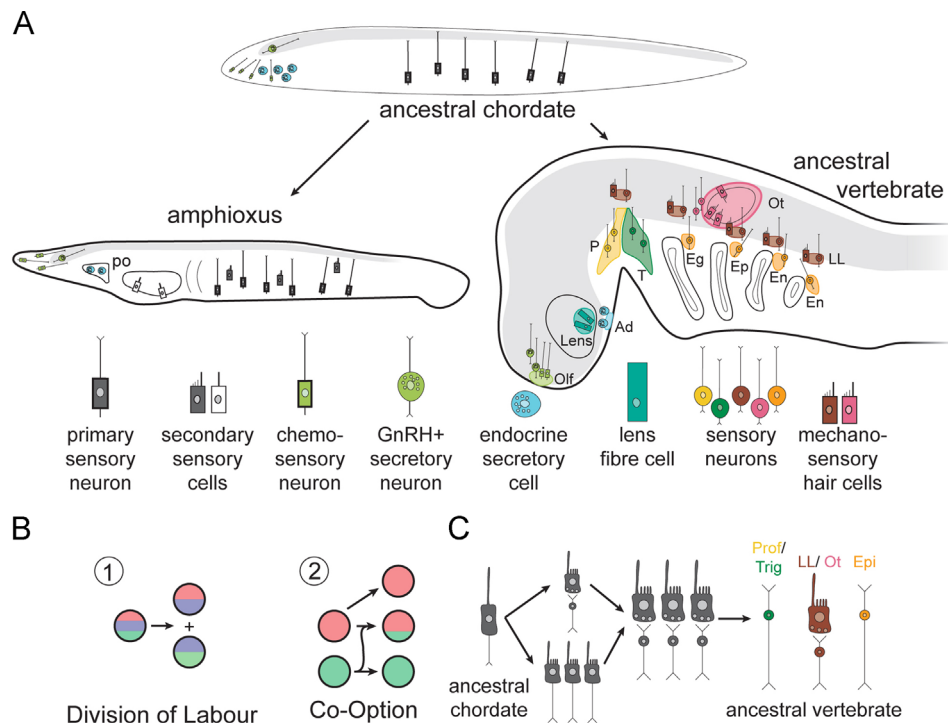


Fig. 4. Proposition of possible scenarios for the evolution of placode derived cell types. (A) Representation of placode-related cell types believed to be present in the ancestor of chordates, and the cell types derived thereof in the descendant cephalochordates and vertebrates. (B) Novel cell types appear in two possible ways. In the process of segregation of function (top), a cell type becomes two sister cell types by splitting part of the transcriptomes between the two daughter cell types. In the process of gene network co-option (bottom), a new cell type originates by merging the transcriptome of two existing cell types. (C) Scenarios for the evolution of somatosensory (SSN: trigeminal/profundal, otic/lateral line) and viscerosensory (VSN: epibranchial) neurons from a putative ciliated primary sensory neuron. In the first scenario (upper pathway), the primary sensory cell splits into a secondary sensory cell and an afferent neuron by segregation of functions. Resulting neurons and sensory cells become three distinct sister cells corresponding to the trigeminal/profundal, otic/lateral line and epibranchial-derived neurons. Alternatively (lower pathway), three sister cell types first evolve as primary sensory neurons. Then the primary sensory cells split into secondary sensory cells and afferent neurons. Finally, in both scenarios, the secondary sensory cells of the trigeminal/profundal and epibranchial, but not otic/lateral line systems are lost.

protein-coupled receptors but they are related to neither insect nor vertebrate olfactory receptors (Robertson, 1998).

In summary, vertebrate and ecdysozoan olfactory chemoreceptors use different receptor proteins and transduction pathways. At present it is not known which of these systems, if any, is ancestral and whether the common ancestor of bilaterians (Urbilateria) had olfactory sensory neurons similar to those of insects or vertebrates. Comparative studies including representatives of the lophotrochozoan clade and more markers of olfactory sensory cells might shed light on this question.

Although the comparison of insect, nematode and vertebrate genomes seemed to indicate that the mammalian type of ORs appeared in vertebrates, true orthologues of ORs have recently been identified in the genomes of invertebrate deuterostomes including amphioxus (Churcher and Taylor, 2009; Niimura, 2009) and the sea urchin (Raible et al., 2006), but not the hemichordate *Saccoglossus* or the tunicate *Ciona* (Churcher and Taylor, 2009; Krishnan et al., 2013).

In protochordates, different types of primary sensory cells are found throughout the ectoderm (amphioxus) or concentrated in palps and tail (ascidians), some of which may have a chemosensory function (reviewed in (Holland and Holland, 2001; Schlosser, 2005)). Three regions in the rostral ectoderm of amphioxus have been suggested to comprise chemosensory cells: the rostrum, the pre-oral pit (precursor to Hatschek's pit) and the circumoral organ. The rostrum is covered with ciliated primary sensory neurons. Similar to the vertebrate olfactory sensory neurons, these cells express one of the > 50 ORs found in the amphioxus genome (Satoh, 2005), arise from a field of ectoderm expressing Pax6 and Six3/6 (Kozmik et al., 2007), and express the POU transcription factor Brn3a (Candiani et al., 2006). For these reasons, these

chemoreceptors are possible homologues of the vertebrate olfactory sensory neurons. Moreover, similar cells are found in the rostral ectoderm of appendicularians (Bollner et al., 1986). The cells of the preoral pit in amphioxus have been proposed to be chemosensory because they are exposed to water flowing into the mouth and carry cilia and microvilli as well as secretory vesicles (Nozaki and Gorbman, 1992; Ruppert, 1997). However, this requires further study since none of these cells has an axon and Hatschek's pit has not yet been shown to be innervated.

Apart from the olfactory sensory cells, the olfactory placode also gives rise to some other cell types, most notably the neurosecretory GnRH cells. Although expression of the neuropeptide GnRH is a defining feature of the GnRH cells migrating from the olfactory placode, GnRH cells are also found in various other locations in vertebrates and might represent diverse cell types. Indeed, each neuron expresses only one of the two to three paralogs GnRH-I–II and –III. Group I GnRH (and group III GnRH, which is only found in teleosts) are expressed in the preoptic area, terminal nerve, and telencephalon while group II GnRH are expressed in more caudal neurons of the midbrain (Whitlock, 2005). While group II GnRH cells are neural plate-derived, the embryonic origin of the other GnRH neurons has been a matter of debate. Recent experiments demonstrated that in birds these GnRH cells originate exclusively in the olfactory placode (Sabado et al., 2012) and suggested that a previous report of a neural crest contribution in mouse may instead reflect leaky expression of the Wnt1-Cre reporter line used in this study (Forni et al., 2011). In contrast, studies in teleost fish involving genetic ablation and tracing suggested that GnRH cells of the septo-preoptic area and terminal nerve arise from the adenohypophyseal placode and neural crest, respectively and not from the olfactory placode

(Whitlock et al., 2003). However, due to the close apposition of the olfactory placode to neural crest and adenohypophyseal placodes in zebrafish embryos, it is possible that the olfactory placode was inadvertently labelled in these experiments and an olfactory origin can, thus, not be ruled out. Regardless of embryonic origin, the localisation and function of GnRH cells is conserved among vertebrates, suggesting that the cell types are homologous.

It is now clear that genes coding for GnRH, NPY and their receptors are found in the genomes of invertebrates (Jékely, 2013; Mirabeau and Joly, 2013; Roch et al., 2011). While GnRH peptides promote reproductive functions in some invertebrates they likely have other functions as well that are currently not well understood (Roch et al., 2011). Phylogenetic analysis as well as experiments assessing the cross-reactivity of GnRH family ligands with various receptors reveal a picture whereby the bilaterian ancestor possessed two related receptors for two distinct peptides, GnRH/corazonin (CRZ) and GnRH/adipokinetic hormone (AKH). Each of those later gave rise to dedicated GnRH receptors as well as to the CRZ receptors of arthropods and AKH receptors of protostomes, respectively. While both types of GnRH receptor are maintained in amphioxus, CRZR-like GnRH receptors have been lost in tunicates and vertebrates (Roch et al., 2011). Moreover, several GnRH genes have been identified in *Ciona* (Roch et al., 2011). While genes encoding GnRH have not yet been identified in the amphioxus genome, a GnRH peptide related to vertebrate GnRH has been isolated in amphioxus (Chambery et al., 2009).

GnRH expressing cells in amphioxus and tunicates have been located in the neural tube and the gonads, and have been shown to modulate reproductive function although they probably serve other functions as well (Castro et al., 2006; Chambery et al., 2009; Kavanaugh et al., 2005; Terakado, 2001). However, apart from some GnRH cells in the ascidian cupular organs no GnRH secreting cells were found in the peripheral nervous system of protochordates or in Hatschek's pit (Castro et al., 2006; Mackie and Singla, 2004; Nozaki and Gorbman, 1992). This suggests that GnRH cells were recruited to the rostral non-neural ectoderm only in vertebrates (see Schlosser, (2005) for further discussion).

Adenohypophyseal placode

The adenohypophysis of the basal vertebrate *Petromyzon* (lamprey) is simpler than that of gnathostomes in that it does not comprise six neurosecretory cell types but only four regions of cells secreting different hormones: GH, ACTH, MSH and the gonadotropin GTH β , suggesting the existence of four adenohypophyseal neurosecretory cell types in the common ancestor of vertebrates (Kawauchi and Sower, 2006). In contrast, no homologues of any of these hormones or their receptors have been identified in the genomes of tunicates or cephalochordates (Dehal et al., 2002; Holland et al., 2008; Putnam et al., 2008) suggesting that all adenohypophyseal cell types evolved in the vertebrate lineage. While some previous studies reported the isolation of proteins related to proopiomelanocortin (POMC) – the common precursor protein for ACTH, MSH and the opioid β -endorphin – from protostomes (Salzet et al., 1997; Stefano et al., 1999), orthologs of the POMC gene, could not be found in the genomes of amphioxus, *Ciona* or any other invertebrate. Because contamination with other animal tissues could not be ruled out in the older protein-sequencing studies this suggests that POMC-derived peptides like the other adenohypophyseal hormones evolved as novel proteins in the vertebrate lineage. A recent analysis, which “dated” the origin of genes specific to placodes or placode-derived organs by phylostratigraphy (Sestak et al., 2013) also found that the vertebrate stem lineage is most enriched for origin events of genes associated with the adenohypophysis, in agreement with the idea that the organ is specific to vertebrates.

This raises the question, from which cell types that existed in the ancestral chordate these vertebrate hormone-secreting cells evolved? While orthologs of the adenohypophyseal hormones have not been found outside of vertebrates, proteins related to each of the three hormone classes – heterodimeric glycoproteins (LH, FSH and TSH), four-helix cytokine-like proteins (prolactin, GH), and neuropeptides (ACTH, MSH) – are present throughout bilaterians (Campbell et al., 2004; Dores and Baron, 2011; Dos Santos et al., 2011; Roch et al., 2011). For example, the POMC gene and its receptor probably evolved by duplication and divergence from opioid and opioid receptor encoding genes in stem vertebrates (Dores and Baron, 2011; Sundström et al., 2010). Although the phylogenetic history of the latter has not been completely resolved, somatostatin/opioid/galanin-type G-protein coupled receptors are probably ancestral bilaterian inventions (Fredriksson and Schiöth, 2005; Mirabeau and Joly, 2013). The glycoproteins LH, FSH and TSH, in turn, probably evolved from the related glycoprotein thyrostimulin, and genes for the two subunits (GPA2, GPB5) of the latter have been found throughout bilaterians (Dos Santos et al., 2009; Park et al., 2005; Sudo et al., 2005). GPA2 and GPB5 are expressed in the CNS of both arthropods and amphioxus, indicating a possible central neural origin of the gonadotropins (Dos Santos et al., 2011; Sellami et al., 2011; Tando and Kubokawa, 2009). Taken together this suggests that the neurosecretory cell types of the adenohypophysis may have arisen as sister cell types from invertebrate neurosecretory cells following gene duplication and functional specialisation.

Neurosecretory cells in chordates were identified in both the CNS of amphioxus and tunicates and in Hatschek's pit of amphioxus (reviewed in Schlosser, (2005)). Previous studies using vertebrate antibodies showed immunolabeling for many neuropeptides and other adenohypophyseal hormones including gonadotropins in Hatschek's pit and, consequently, suggested this to be a neurosecretory organ homologous to the vertebrate adenohypophysis (reviewed in Schlosser (2005)). However, since the genome sequencing of both *Ciona* and amphioxus has revealed the absence of adenohypophyseal hormones in these taxa, it is most likely that previously observed immunoreactivity for adenohypophyseal hormones in Hatschek's pit reflects cross-reactivity with other but possibly related molecules such as thyrostimulin, which is transiently expressed in the anterior endodermal gut diverticulum proposed to contribute to Hatschek's pit (Dos Santos et al., 2009).

While many transcription factors imparting the regional identity of the adenohypophyseal placode are shared by the olfactory and/or lens placode, transcription factors involved in the specification of different lineages of endocrine cells are more specific. Prop1 and Pit1 are expressed in the lineage of the somatotropes, lactotropes and gonadotropes, whereas Tbx19 is expressed in the corticotropes and melanotropes (Liu et al., 2001). Phylogenetic analysis of the Tbx gene family suggests that Tbx19 arose by duplication of Brachyury at the base of chordates (Belgacem et al., 2011), but no Tbx19 gene has been reported in amphioxus. However, expression of the POU-family transcription factor Pit1 in amphioxus is restricted to Hatschek's pit (Candiani et al., 2008).

Taken together with the immunoreactivity for various hormones and the presence of secretory vesicles in Hatschek's pit this suggests that the latter probably acts as a neurosecretory organ, with release of secretory vesicles being triggered by environmental cues (Nozaki and Gorbman, 1992). With the subsequent evolution of adenohypophyseal hormones the functions of this organ may have diversified and its neurosecretory cells may have duplicated and diverged to form many new and specialized sister cell types. After the recruitment of other cell types such as primary sensory cells and GnRH cells this neurosecretory organ may have given rise to a rostral placode, from which subsequently adenohypophyseal and olfactory placodes evolved (see Schlosser (2005) for a detailed scenario).

Despite the novelty of the specific neurosecretory cell types found in the vertebrate adenohypophysis, several types of scattered neurosecretory cells have been found in the PNS and CNS of cnidarians and many bilaterians indicating that neurosecretory cells are ancient cell types (Hartenstein, 2006; Tessmar-Raible, 2007). However, how neurosecretory cells diverged and diversified in evolution remains to be elucidated. Recently, several parallels have been drawn between the neurosecretory cells of the adenohypophysis and the corpora cardiaca or corpora allata of arthropods (De Velasco et al., 2004; Hartenstein, 2006; Wirmer et al., 2012). These hormone producing glands also develop anteriorly and receive neural input from neurosecretory cells of the pars intercerebralis and lateralis of the protocerebrum, which in turn have been proposed to be homologous to the neurosecretory cells of the hypothalamus based on their molecular signature and shared expression of the neuropeptide RFamide (De Velasco et al., 2007; Tessmar-Raible et al., 2007). At the level of cell types, a further similarity between adenohypophysis and corpora allata is the secretion of nitric oxide with an autocrine effect on hormone secretion (Wirmer et al., 2012). Finally, both adenohypophysis and the corpora allata express related cytochrome P450 enzymes that catalyse the synthesis of bioactive fatty-acid related compounds, which act as hormones in insects (juvenile hormone, the main hormone secreted by the corpora allata) or stimulate hormone release in the adenohypophysis (Wirmer et al., 2012). While these similarities suggest that neurosecretory cells of corpora allata (and/or corpora cardiaca) and adenohypophysis may be derived from a common subtype of bilaterian neurosecretory cells, more data are needed to confirm this and to elucidate, whether and where homologous cell types can be found in amphioxus and other animal phyla.

Lens placode

The lens fibre cells have no obvious counterpart in invertebrates. A defining feature of lens fibre cells is expression of crystallins. A single $\beta\gamma$ -crystallin gene was identified in the tunicate *Ciona* and its expression localised to the palps and the otolith cells in the neural tube. Swapping the promoter of *Ciona* and *Xenopus* in reporter construct assays showed that the regulatory landscape of the vertebrate lens is similar to that of the expression domain in *Ciona*. This suggests that not only the crystallin was recruited to the lens region but also the regulatory network for its expression (Shimeld et al., 2005).

The cell type which co-opted the $\beta\gamma$ -crystallin sub-network is not known, but the fact that the lens placode expresses *SoxB1* genes during development (Kamachi et al., 1998) and the lens fibre cells bear resemblance with neurons (Frederikse et al., 2012) suggests that the lens fibre cells might be modified neurons.

Profundal and trigeminal placodes

At present, the only known molecular difference between the profundal and the trigeminal neurons is the expression of *Pax3* in the former (Baker et al., 1999). The origin of the difference between the profundal and trigeminal cells is obscure, although even the most basal vertebrates (i.e. lampreys) possess distinct profundal and trigeminal ganglia (Kuratani et al., 1997).

Besides, the trigeminal somatosensory neurons (SSNs) are very similar to the neural crest-derived neurons of the dorsal root ganglia (DRG). A micro-array study showed that trigeminal and DRG ganglia exhibit virtually identical transcriptomes, although the *Brn3a* target genes differ slightly between the two tissues (Dykes et al., 2010; Eng et al., 2007). However, the trigeminal ganglion contains a mixture of placode- and neural crest-derived somatosensory neurons (D'Amico-Martel and Noden, 1983; Quina

et al., 2012), and the latter are expected to be similar to the neural crest-derived DRGs. The molecular differences between neural crest-derived and placode-derived trigeminal neurons are at present largely unknown but differences in function and peripheral targets do exist.

SSNs of the trigeminal and profundal ganglia (as well as those of the neural crest-derived DRGs and superior/jugular ganglia of nerves IX and X) express the somatosensory marker *Brn3a* as well as its target *DrgX*. *Brn3* paralogs and *DrgX* are also expressed in vestibulocochlear and lateral line afferents (Rebelo et al., 2007). Together with *Isl1* (a gene expressed in somatic but not visceral sensory neurons) expression of *Brn3* and *DrgX* homologues has been used to trace the origin of somatic sensory neurons back to the bilaterian ancestor. Indeed in the molluscs *Aplysia*, *Lymnaea* and *Sepia*, mechanoreceptors express the same somatic sensory signature (Nomaksteinsky et al., 2013).

Are there trigeminal-like somatosensory neurons in invertebrate chordates? The epidermal sensory neurons that get born in the ventral ectoderm in amphioxus are possible homologues, as they express *Brn3* (Candiani et al., 2006). However, they also express *Tlx* (Lu et al., 2012), a gene related to chicken *Tlx1* and *Tlx3*, which collectively are expressed not only in the trigeminal ganglion but also in the three epibranchial-derived ganglia and in the vestibulocochlear ganglion (Logan et al., 1998). Moreover, they are believed to be related to the epidermal sensory neurons derived from the ventral midline of ascidians (Lu et al., 2012), which have recently been shown to share molecular similarities with otic/lateral line mechanoreceptors (see below; Tang et al., 2013). Interestingly, *Pax3/7* does not seem to be expressed in the ectodermal sensory neurons of amphioxus (Holland et al., 1999), but it is expressed in dorsal ectodermal cells of ascidians (Mazet et al., 2003; Wada et al., 1997). Taken together this suggests that there is no one-to-one correspondence between the epidermal sensory neurons of protochordates and either SSNs or viscerosensory neurons (VSNs) of vertebrates. One possibility is that amphioxus epidermal sensory neurons corresponding to both SSNs and VSNs are derived from an ancestral somatosensory cell (see below). Alternatively, sub-populations of amphioxus epidermal sensory neurons may represent the different cell types that diverged into SSNs, lateral line/vestibulocochlear neurons and VSNs in vertebrates. In support of this idea, certain genes are expressed only in subsets of epidermal sensory neurons, including *Hox3*, *Hox4*, *Six1/2* and *Eya* (Kozmik et al., 2007; Schubert et al., 2004). An amphioxus *SoxB1* gene is expressed in epidermal sensory neurons at intermediate AP level, suggesting these cells might correspond to the *SoxB1*-expressing epibranchial, lateral line and otic placode-derived neurons (Meulemans and Bronner-Fraser, 2007), whereas the *SoxB1*-negative epidermal sensory neurons might correspond to the SSNs derived from the *SoxB1*-negative trigeminal placode. Another argument against the homology of amphioxus epidermal sensory neurons and profundal or trigeminal neurons is that the former are ciliated primary sensory neurons while the latter are not ciliated and sense stimuli with free terminal endings.

Similar to the dorsal root ganglia, the trigeminal and profundal ganglia contain different sub-populations of somatosensory neurons, with each class expressing a different neurotrophin/growth factor receptor. Mechanoreceptors with Ruffini endings and those innervating Pacini corpuscles express *TrkB*, large mechanoreceptors express *TrkC* and *Runx3*, and nociceptors express *TrkA* and *Runx1*, at least at developmental stages (Marmigere and Ernfrors, 2007). The fact that only a single *Trk* gene and a single *Runx* gene are found in several invertebrate genomes has led to the interesting speculation that the cell types associated with the different sensory modalities evolved with the two rounds of whole genome duplication at the stem of vertebrates (Benito-Gutierrez et al., 2005).

Lateral line and otic placodes

The fact that otic and lateral line placodes give rise to similar cell types has led to several proposals of their common origin. In its initial form, the acousticolateralis hypothesis suggested that the inner ear is a specialisation of the lateral line system (Jørgensen, 1989). We now know, however, that all living vertebrates possess distinct lateral line and otic systems (Popper and Fay, 1997). Therefore, the similarities between the otic and lateral line cell types rather reflect independent evolution from a common cell type, which was probably ciliated and mechanosensory.

Regardless of their evolutionary relationship, ciliated mechanoreceptors sensing sounds and movements are very old (Fritzsche et al., 2010) and share regulatory networks for specification. Ciliated sensory neurons in protostomes such as the neurons associated with bristles in *Drosophila* share developmental mechanisms with the vertebrate otic hair cells. Thus, the bilaterian ancestor had mechanoreceptors regulated by genes of the bHLH family such as *Ngn* and *Atonal*, but the morphology of mechanoreceptor has diversified a lot in the protostome and deuterostome lineages (Fritzsche et al., 2002, 2007).

The specification of olfactory, photosensory and mechanosensory cells relies on a deeply conserved pathway involving sequential activation of three transcription factors: PaxB (Pax2 or Pax6), *Atonal* and *Pou4F/Brn3* (Fritzsche et al., 2005). It should be noted, however, that not all mechanosensory cells are specified by *Atonal* expression; some express *Achaete-scute*, another bHLH transcription factor, and this dichotomy dates back to cnidarians (Fritzsche et al., 2005). Expression of an *Atonal* gene is characteristic of, and required for the generation of, mechanoreceptors in both *Drosophila* and vertebrates (Bermingham et al., 1999; Jarman et al., 1993). In agreement, it was shown in a recent study that in addition to expressing *Brn3*, epidermal sensory neurons in the ventral midline of *Ciona* deploy a similar pathway to that specifying hair cells in vertebrates, including regulation of *Brn3* by *Atonal* (Tang et al., 2013). In vertebrates, this *Atonal* – *Brn3* pathway is involved in the specification of olfactory sensory neurons, retinal ganglion cells and hair cells. The role is conserved in mechanosensory cells in *C. elegans* and in photoreceptors and chemoreceptors in *Drosophila*, suggesting an ancestral role of the *Atonal* – *Brn3* pathway in all three systems (Certe et al., 2000; Finney and Ruvkun, 1990; Zhang et al., 2006). The epidermal sensory neurons of amphioxus and *Ciona* might thus represent homologues of the ciliated mechanoreceptors of protostomes and vertebrates. In line with this idea, epidermal sensory neurons in both amphioxus and *Ciona* are ciliated and are thought to be predominantly mechanoreceptors (Crowther and Whittaker, 1994; Pasini et al., 2006).

Among many phyla studied, vertebrates are the only ones where ciliated mechanosensory cells are represented only by secondary sensory cells. Tunicates, cephalochordates and molluscs all have both primary sensory neurons provided with an axon and secondary mechanoreceptors, whereas cnidarians have only primary mechanoreceptor neurons (Burighel et al., 2011). This pattern suggests that during the course of evolution, the hair cells and the neurons innervating them appeared by division of labour, with the hair cell assuming the sensory function and the afferent neuron taking on the neuronal function (Fritzsche et al., 2002, 2007). In support of this hypothesis, during development of the inner ear in vertebrates, the hair cells and vestibulocochlear neurons share a common progenitor expressing the proneural gene *Ngn1*, while the bHLH transcription factor *Atonal* characteristic of mechanosensory neurons is upregulated in the differentiating hair cell as *Ngn1* is downregulated (Bell et al., 2008; Satoh and Fekete, 2005). This mechanism is reminiscent of a Haeckelian recapitulation.

Besides the epidermal sensory neurons, other populations of ciliated mechanoreceptors have been identified in invertebrate

chordates. In tunicates, secondary hair cells and their afferent neurons are located around the mouth, in the coronal organ of ascidians and thaliaceans and in the circumoral organ of appendicularians. A recent study comparing the ultrastructure of hair cells in the three major groups of tunicates concludes that in the ancestral tunicate hair cells had a single kinocilium as found in vertebrate hair cells (Rigon et al., 2013). However, the coronal organ is derived from the stomodeal primordium, a region with putative homology to the rostral placodes as discussed above. The hair cells of the coronal organ in ascidians are believed to be homologous to mechanoreceptors of the oral spine in amphioxus (Lacalli, 2004).

Ciliated mechanoreceptors harbouring their own axons are present in the atrium of ascidians. These primary sensory cells are better candidate homologues of the otic and lateral line hair cells, because they derive from a Pax2⁺ Foxl⁺ region similar to the vertebrate caudal placodes (Gasparini et al., 2013; Mazet et al., 2005). Under this hypothesis, multiple scenarios are possible for the evolution of vertebrate ciliated mechanoreceptors. First, the hair cells produced by the otic and lateral line placodes evolved from a primary sensory neuron in the ancestors of chordates or the tunicate-vertebrate clade, and the secondary hair cells represented by the coronal/circumoral organ in tunicates have been lost in vertebrates. Alternatively, the primary sensory cells were lost and the secondary hair cells and their afferents, originally appearing in the rostral proto-placode, were relocated to be generated by the caudal proto-placode. A third possibility is that in the common ancestor both regions made both primary and secondary sensory cells, with reciprocal loss explaining their distribution in current lineages. Distinguishing between these hypotheses will require a significant improvement in our understanding of the evolutionary relationships between vertebrate and invertebrate sensory cells.

The otic placode also generates a number of support cell types, although the evolution of these cells has been much less studied than that of hair cells. Interestingly, similar to vertebrates, the mechanoreceptor cells and the support cells in the bristle and chordotonal organs of *Drosophila* develop clonally from a common progenitor by a Notch-mediated mechanism.

Some vertebrates are also endowed with a particular type of lateral line placode-derived cells that resemble a lot the lateral line mechanoreceptor but sense electrical fields. Since these are found in both bony fishes, cartilaginous fishes and agnathans, it seems likely that the vertebrate ancestor had both mechanosensory and electrosensory lateral line systems (reviewed in Baker et al., 2013). However, electroreceptor cells related to mechanoreceptors have not been described in invertebrates and may have evolved as sister cell types of mechanoreceptors in stem vertebrates (Baker et al., 2013).

Epibranchial placodes

Similar to visceromotor neurons, the VSNs originating from the epibranchial placodes express the pan-visceral marker *Phox2*. The evolution of *Phox2*-expressing cells has recently been investigated and, somewhat surprisingly, no *Phox2*-expressing sensory cells have been found outside of vertebrates, at least not in the peripheral nervous system (Nomaksteinsky et al., 2013). This suggests that the viscerosensory neurons are a vertebrate novelty, possibly having its origin in the recruitment of the homeodomain transcription factor *Phox2* turning a *Brn3*-positive somatic cell type into a *Brn3*-negative visceral neuron. In line with this idea, loss of *Phox2b* function in mouse results in a switch from visceral to somatic phenotype, showing that *Brn3*-positive somatic character is a ground state onto which *Phox2* superimposes a visceral identity (D'Autr aux et al., 2011).

Evolution of somatosensory and viscerosensory placode cell types

At the level of cell types, the olfactory sensory neurons and neuro-endocrine cells of the adenohypophysis are distinct from other placode-derived cells and likely homologous chemosensory and neurosecretory cells can be found in invertebrates (see above). In contrast, the evolutionary relationship between somatosensory neurons (cutaneous SSN as in the trigeminal ganglion as well as vestibulocochlear/lateral line neurons) and viscerosensory neurons derived from the epibranchial placodes is less straightforward. Shared developmental origin would suggest a common ancestry of the mechanoreceptive vestibulocochlear/lateral line neurons and viscerosensory neurons, whereas expression of *Brn3* and *DrgX* and “somatic” function suggest a common origin of trigeminal-profundal and mechanoreceptor neurons. This suggests that these 3 cell types may be sister cell types but the sequence, in which they split from each other is unclear and the available data in invertebrates are compatible with different scenarios. In addition, as discussed above, the secondary sensory receptors of the otic and lateral line placodes (hair cells) and the neurons that innervate them have been suggested to be sister cell types and derive from an ancestral ciliated primary mechanoreceptor (Fritzsche et al., 2002, 2007). It has been proposed that viscerosensory neurons evolved similarly by cell type duplication and that the associated secondary sensory receptor cell was subsequently lost (Baker, 2008). A parallel scenario may account for the origin of trigeminal somatosensory neurons. However, it is currently unclear whether the split into secondary receptor cells and sensory neurons preceded the diversification of sensory neurons/receptors or vice versa (Fig. 4C). The available evidence in amphioxus and ascidians does not allow to recognise unequivocal homology of vertebrate cell types with particular populations of ciliated primary sensory neurons, and, thus, the differences between trigeminal and otic/lateral line SSNs and VSNs may have arisen in the vertebrate lineage. According to this scenario, Tlx expression may have been kept in all the vertebrate cranial ganglia, while *Brn3* has been lost in the VSNs which gained expression of *Phox2b*. Future comparative studies of gene expression might shed more light on the evolutionary relationship of these cell types.

Summary and conclusions

In this first review we have focused on the evolution of the cell types that develop from placodes. In considering the diversity of these cells alongside the mechanisms that underlie their specification and differentiation, and comparing these to what is known about the development of similar cells in amphioxus, tunicates and other invertebrates, we have tried to construct a framework for interpreting these different levels of evidence and assess models for placode cell type origins. While the evolution of some cell types remains enigmatic, hypotheses emerge for others that allow us to make testable predictions concerning the development, function and molecular signature of cell types in invertebrates. To test these models, and to understand the evolution of other placode cell types, further study is needed. Specifically we propose that characterisation of vertebrate placode cells needs to move beyond candidate gene approaches and consider the cassettes of genes that are responsible for both differentiation and physiological function. Transcriptional profiling offers a potential route to uncovering these on a genome-wide basis. Such studies will need to be accompanied by similar studies on sensory cell populations in invertebrates, most urgently those in the invertebrate chordates. Ideally such studies would be complemented by physiological assessment of the types of sensation these cells

convey, as well as knowledge of their lineage and the developmental mechanisms by which they are patterned and specified. While recent progress has been made on the latter and the development of robust mechanisms for experimentally manipulating some invertebrate embryos (for example Abitua et al. (2012), Lu et al. (2012)) promises further insight, direct physiological assessment of many invertebrate sensory cells is likely to remain challenging.

In the second part of our review (Schlosser et al., this issue), we extend our consideration of placode evolution to consider the patterning mechanisms that underlie the regionalisation of the ectoderm, specification of the pre-placodal region and subsequent formation of individual placodes. We end this second review with a synthesis of our conclusions from both papers, including a new scenario for the stepwise evolution of the many genetic, cellular and developmental characters which in combination make placodes a fascinating example of an evolutionary novelty whose origin in the vertebrate stem lineage now marks such a fundamental difference in the cephalic sensory systems of vertebrates and invertebrates.

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